The oncogenic and immunological roles of histidine triad nucleotide-binding protein 1 in human cancers and their experimental validation in the MCF-7 cell line

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Background: Histidine triad nucleotide binding protein 1 (*HINT1*) is a haplo-insufficient tumor suppressor gene that plays a significant role in cell proliferation and survival. However, to date, no systematic pan-cancer analysis has been conducted to explore its function in prognosis, and its oncogenic and immunological roles. We also analyzed the role of *HINT1* in breast cancer (BC) progression *in vitro*.

Methods: An analysis of the *HINT1* expression pattern was performed using the TIMER database. The infiltration of immune cells into several cancer types was also studied using the Xena Shiny tool. To determine the relationship between stemness and the expression of *HINT1* mRNA, the Spearman correlation test was used with the SangerBox tool. The correlation between *HINT1* and functional states in various cancers was determined from the CancerSEA database. The potential role of *HINT1* in BC oncogenesis was also investigated by Western blot and Annexin V/PI assays.

Results: The Cancer Genome Atlas pan-cancer data analysis suggested that *HINT1* was extensively altered in most tumor tissues but not in most adjacent normal tissues. A high expression of *HINT1* was associated with the decreased infiltration of cluster of differentiation (CD)4$^+$ T cells. Importantly, increased *HINT1* expression was also associated with a large majority of tumors with high stemness and lower stromal, immune, and estimate scores. Further, the expression of *HINT1* was significantly associated with the tumor mutational burden (TMB) and microsatellite instability (MSI) in certain tumor types. Finally, *HINT1* overexpression was found to impair BC progression by promoting cell apoptosis. *HINT1* upregulation also reduced the expression of microphthalmia transcription factor (*MITF*) and β-catenin in BC Michigan Cancer Foundation-7 (MCF-7) cells, and the phosphorylation of protein kinase B (p-Akt).

Conclusions: The present study showed that *HINT1* plays an oncogenic role in various cancers and could also be used as a biomarker for BC.

Keywords: Histidine triad nucleotide binding protein 1 (*HINT1*); breast cancer (BC); oncogenic role; tumor microenvironment; apoptosis

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Introduction

Histidine triad nucleotide-binding protein 1 (*HINT1*) belongs to the histidine trimer family (1,2). *HINT1* has high levels of conserved expression across multiple body tissues. The three branches of the *HIT* family are histidine triad nucleotide-binding proteins, fragile histidine triad (*FHIT*), and galactose-1-Puridylyltransferase. Several studies have shown that *FHIT* is a tumor suppressor, and the expression of *FHIT* is associated with preneoplastic and malignant disorders early in the disease process (3,4). Accordingly, it was presumed that *HINT1*, which is structurally homologous to *FHIT*, would act similarly as a tumor suppressor protein. There is increasing evidence that *HINT1* is a haplo-insufficient tumor suppressor gene that plays a significant role in cell proliferation and survival (5). Additionally, normal *HINT1* expression is closely linked to poor tumor differentiation, which suggests that *HINT1* could generally be used as a tumor suppressor (6-8).

HINT1 appears to have an inhibitory effect on gene transcription pathways. For example, *HINT1* interacts with the basic helix-loop-helix microphthalmia-associated transcription factor (*MITF*), which inhibits its transcriptional regulating activity on oncogenes (6). Genovese *et al.* confirmed that *HINT1* significantly inhibits both *MITF* and β-catenin transcriptional activities in melanoma cell lines (9). Moreover, *HINT1* affects hepatocellular cancer migration and invasion *in vitro* by modulating protein kinase B (Akt) expression and phosphorylation (10).

Highlight box

Key findings

- *HINT1* plays an oncogenic role in various cancers and could also be used as a biomarker for BC.

What is known and what is new?

- TCGA data suggested that *HINT1* was extensively altered in most tumor tissues but not in most adjacent normal tissues. A high expression of *HINT1* was associated with the decreased infiltration of $CD4^+$ T cells. Further, the expression of *HINT1* was significantly associated with the TMB and MSI in certain tumor types.
- We found that *HINT1* overexpression impaired BC progression by promoting cell apoptosis. *HINT1* upregulation also reduced the expression of *MITF* and β-catenin in BC MCF-7 cells, and the phosphorylation of Akt.

What is the implication, and what should change now?

- *HINT1* may be a useful biomarker for breast cancer.

Breast cancer (BC) is the most frequently diagnosed cancer, and the second largest cause of cancer-related deaths in women (11,12). Due to early prevention, a 5-year relative survival rate of >90% for BC patients has been achieved in some developed countries (13). As BC is a metastatic cancer and currently incurable, it is very likely that the disease will spread to distant secondary organs (such as the bone, liver, lung and brain) (14,15). The development of novel diagnostic and therapeutic markers for breast cancer may provide more information about its pathogenesis and progression (16).

In the last decade, significant progress has been made in understanding BC and developing prevention methods. The pathogenesis and tumor drug resistance mechanisms were revealed following the discovery of BC stem cells, and a number of genes have been found to be related to BC disease (17,18). Due to the heterogeneity of cancers, it is crucial that we gain a better understanding of the genesis of BC and develop new therapeutic strategies.

This study sought to determine the mechanisms and functions of *HINT1* and its interaction molecules in cancer, and their role in a variety of cancer types. We examined the relationships between *HINT1* expression and immunotherapy in different cancers, and their immune microenvironments. We also used single-cell sequencing data to investigate the relevant cancer cell status for *HINT1*. Finally, it analyzed the role of *HINT1* in BC progression *in vitro*. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6637/rc).

Methods

Gene expression

An analysis of the *HINT1* expression pattern was performed using the TIMER database (https://cistrome.shinyapps.io/timer/) (19,20). Using the DifferExp module, we compared the gene expression of tumors and adjacent normal tissues across all The Cancer Genome Atlas (TCGA) tumors. The box plots show the gene expression levels, and the Wilcoxon test was used to determine differential expression significance. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Immune cell infiltration

The infiltration of immune cells into several cancer types was studied using the Xena Shiny tool (https://shiny.hiplot.

com.cn/ucsc-xena-shiny/). TCGA: Associations between Molecular Profile and Tumor Immune Infiltration module of Xena Shiny was used to examine the correlations between the mRNA expression levels of HINT1 in 6 immune cell types and tumor infiltration in 33 TCGA tumor samples.

Tumor immune microenvironment and immunotherapy

To determine the relationship between stemness and the expression of *HINT1* mRNA, the Spearman correlation test was used with the SangerBox tool (http://vip.sangerbox.com/home.html). This pan-cancer study analyzed tumor stemness using RNA stemness scores (RNAss) based on mRNA expression and deoxyribonucleic acid stemness scores (DNAss) based on DNA methylation patterns. The tumor mutational burden (TMB) measures the number of mutated bases per megabase and was used to evaluate the immunotherapy response (21). Additionally, microsatellite instability (MSI) status was a biomarker for the selected immunotherapy group (22). Based on the Xena Shiny database, we used TCGA: Associations between Molecular Profile and TMB/Sremness/MSI (Radar Show) module to examine the link between *HINT1* mRNA expression levels and stemness, TMB, and MSI in 33 TCGA tumors, and the "fmsb" package was used to visualize these findings. The tumor microenvironment (TME) contains stromal and immune elements that are positively correlated with stromal and immune scores. The estimate score, which is a composite score based on stromal and immune scores, provides an insight into the relative ratio of stromal and immune components within the TME. We further calculated the immune, estimate, and estimate scores using the "estimate" and "limma" packages to examine the correlation between *HINT1* mRNA expression and stromal function.

Cancer single-cell functional status

The CancerSEA database (http://biocc.hrbmu.edu.cn/CancerSEA/) reveals the distinct functional states of specific genes for different cancer types at the single-cell level, and avoids the limitations caused by tumor heterogeneity (23). The correlation between HINT1 and functional states in various cancers was determined from the CancerSEA database, which is available for download. We then created a correlation heatmap using the R package "ggplot2" to compare *HINT1* to the 14 functional states of cancer cells.

Cell cultures and the transfection of the HINT1 overexpression plasmid

The Michigan Cancer Foundation-7 (MCF-7) BC cell line was purchased from the National Collection of Authenticated Cell Culture in Shanghai. The cells were cultured in medium with 10% fetal bovine serum (BI, Israel) and 1% penicillin-streptomycin mixture (Solabrio, China) at 37 ℃ in a 5% carbon dioxide incubator. Transfection with a HINT1 overexpression plasmid consisted of seeding 5×10^5 cells into 6-well plates, covering 50–70% of the bottom surface areas of the wells, and culturing the cells overnight at 37 ℃ in complete medium. The mock and *HINT1* overexpression plasmids were designed and synthesized by the Riobio Co., Ltd. (Guangzhou, China). In accordance with the manufacturer's instructions, the plasmids were transfected using Lipofectamine 3000 reagent (Thermo Fisher, China). Next, the transfected cells were cultured at 37 ℃ for 24 h, after which Western blotting was used to evaluate the transfection efficacy.

Western blot

The total protein was extracted using ristocetin-induced platelet aggregation lysis buffer (Solabrio, China). A bicinchoninic acid kit (Thermo Fisher, China) was used to measure the total protein concentration. The total protein was denatured by being boiled in the loading buffer (CWBIO, China) at 100 ℃ for 10 minutes, and was then loaded onto a 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis gel. The proteins were thoroughly separated, then transferred to polyvinylidene fluoride membranes, blocked with 5% non-fat milk at room temperature for 1 h, and subsequently cultured with *HINT1* (1:1,000, Abcam, China), *MITF* (1:1,000, Abcam, China), Akt (1:1,000, Cell Signaling Technology, USA), p-Akt (1:1,000, Cell Signaling Technology, USA), β-catenin (1:2,000, Proteintech, USA), or glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:20,000, Proteintech Group, USA) at 4 ℃ overnight. After being washed 3 times with Tris Buffered Saline with Tween (T-BST), the membranes were incubated with horseradish peroxidase-conjugated antibodies (1:5,000, Affinity Bioscience, China) for 1 hour at room temperature. For chemiluminescence, the membranes were subsequently washed 3 more times with T-BST, and enhanced chemiluminescence reagent (NCM, China) was applied under the visualizer. All the experimental

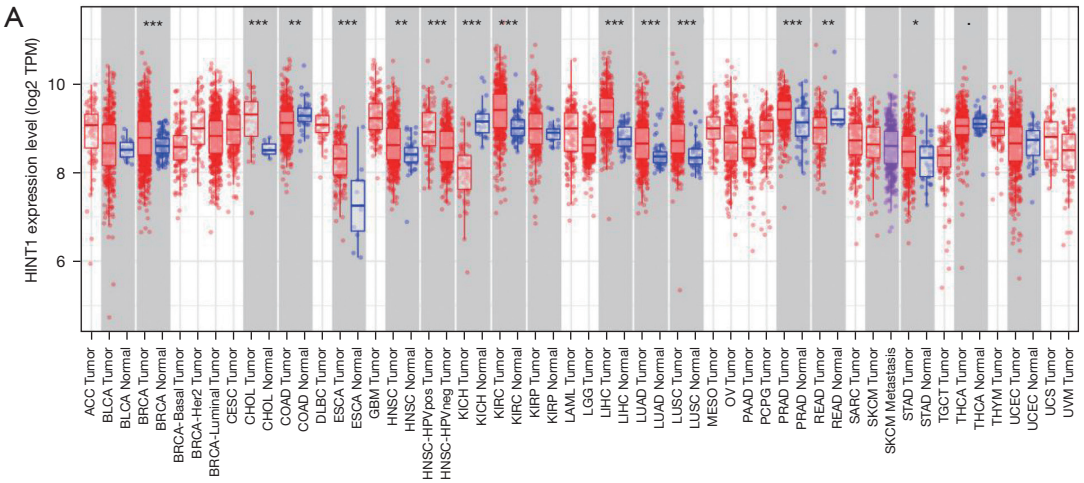

Figure 1 The expression of *HINT1* extensively changed in pan-cancers. *, P<0.05, **, P<0.01, ***, P<0.001.

procedures were repeated 3 times.

Annexin V/PI assays

The Annexin V-FITC/PI kit (BD, USA) was used to analyze the effects of cell apoptosis. Briefly, the cells that had been successfully transfected with the *HINT1* overexpression plasmids or mock plasmids were digested, taken out of the 6-well plate and resuspended with 1× binding buffer at a concentration of 1×10^6 cell/mL. Next, 100 μL of the cell solution was transferred into a culture tube along with 5 μL of FITC-Annexin V and 5 μL of PI. After gentle vortexing, the cells were incubated with the reagents for 15 min at room temperature in darkness, after which 400 μL of 1× binding buffer was added, and the final solution was analyzed by flow cytometry (BD, Calibur).

Statistical analysis

The correlations were analyzed using Spearman's correlation test or Pearson's correlation test. The Cox proportional hazards model was used to calculate the survivorship risk and HR. Each of the variables was evaluated using the K-M survival plot and compared using a log-rank test. As 2 sets of results were detected, a significance level of 0.05 was set.

Results

HINT1 expression changed extensively in different cancers

The *HINT1* gene expression levels were measured in 33 cancer types analyzed by TCGA pan-cancer database. When compared to the adjacent normal tissues, *HINT1* mutations tended to be extensive and a statistically significant difference was found in half of all the pan-cancers. *HINT1* was highly expressed in breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), esophageal adenocarcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), and stomach adenocarcinoma (STAD), and poorly expressed in colon adenocarcinoma (COAD), kidney chromophobe (KICH), and rectum adenocarcinoma (READ), and the difference was statistically significant (*Figure 1*).

Associations between HINT1 mRNA expression and immunotherapy and immune microenvironment in pan-cancers

The scatterplot analysis showed that the *HINT1* gene

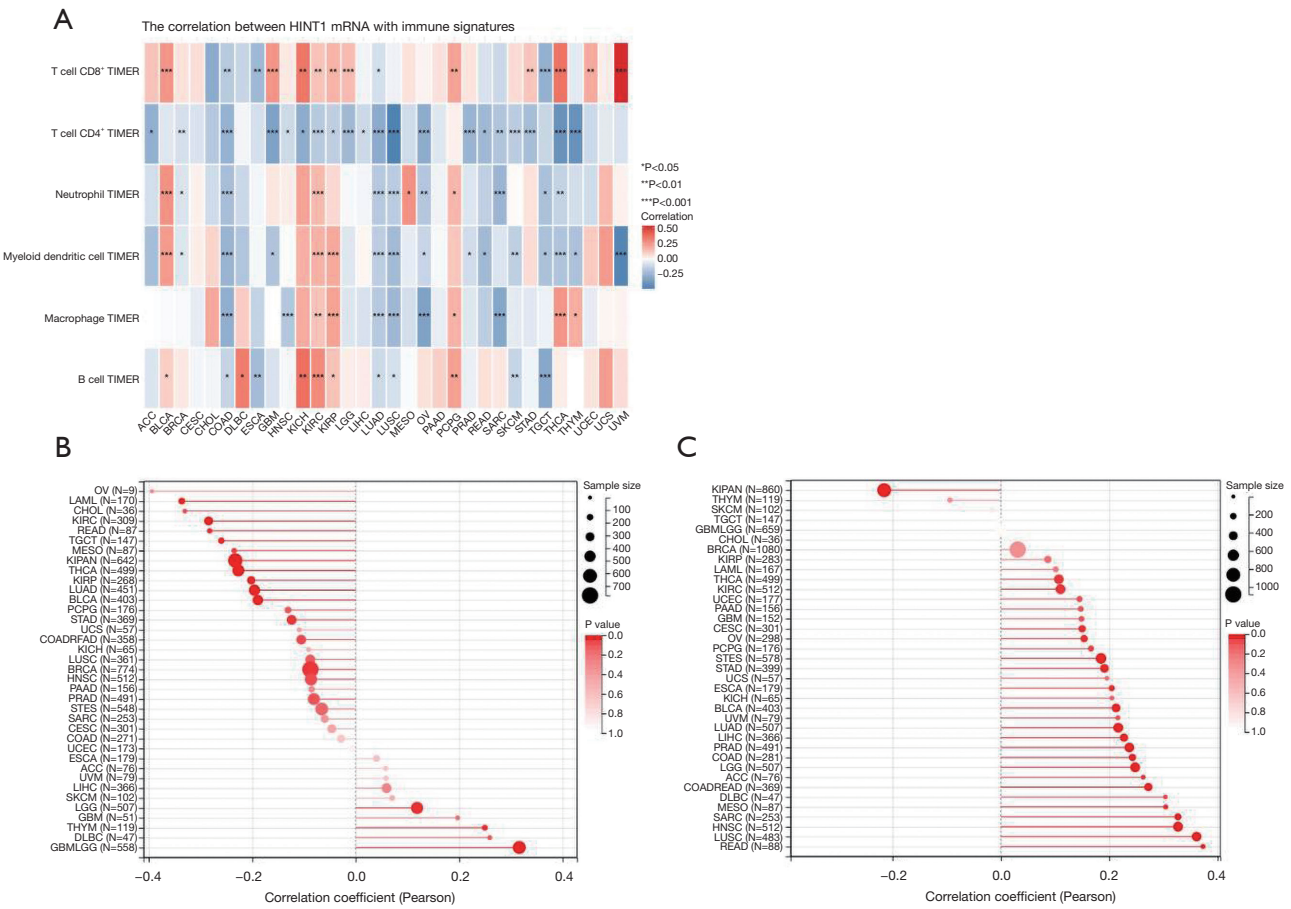

Figure 2 The correlation between the mRNA expression of *HINT1* and tumor infiltration, and the relationship between tumor stemness and the expression of *HINT1* mRNA. (A) The correlation between the mRNA expression of *HINT1* in 6 immune cell types and tumor infiltration in 32 TCGA tumor samples. (B) The relationship between DNAss and the expression of *HINT1* mRNA. (C) The relationship between RNAss and the expression of *HINT1* mRNA.

coefficient was correlated with cancer infiltration in each cell type analyzed in diverse cancer types. The expression of *HINT1* was negatively correlated with cluster of differentiation (CD)4$^+$ T cell infiltration in a large majority of the examined tumors (*Figure 2A*).

The DNAss showed that the expression of *HINT1* was positively correlated to GBMLGG, LGG, and thymoma (THYM), and negatively correlated to LUAD, colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma (COADREAD), LAML, BRCA, KIRP, KIPAN, STAD, KIRC, thyroid carcinoma (THCA), MESO, READ, testicular germ cell tumors (TGCT), bladder urothelial carcinoma (BLCA), and CHOL (*Figure 2B*). The RNAss showed that the expression of *HINT1* was positively associated with LGG, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), LUAD, COAD, COADREAD, ESCA, stomach and esophageal carcinoma), SARC, STAD, PRAD, HNSC, KIRC, LUSC, LIHC, THCA, MESO, READ, ovarian serous cystadenocarcinoma (OV), pheochromocytoma and paraganglioma (PCPG), BLCA, adrenocortical carcinoma (ACC), and lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), while the expression of *HINT1* was negatively associated with KIPAN (*Figure 2C*).

A positive correlation between *HINT1* mRNA expression and stemness was observed in BLCA, BRCA, CESC, COAD, DLBC, ESCA, GBM, HNSC, KIRC, LGG, LIHC, LUAD, LUSC, OV, PCPG, PRAD, SARC, SKCM, STAD, and THCA, while a negative correlation was observed in KICH (*Figure 3A*, Table S1). *HINT1* was

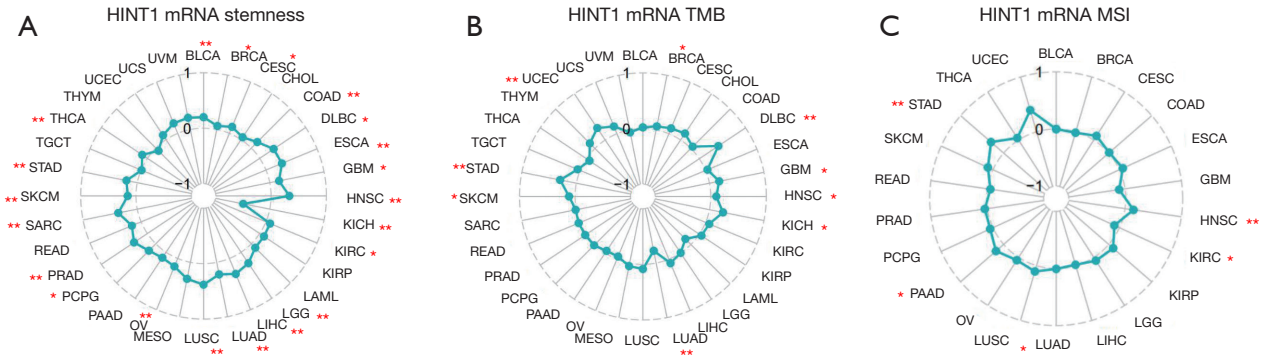

Figure 3 The correlation between the HINT1 mRNA expression and stemness, TMB, and MSI in 32 TCGA tumors. (A) Stemness. (B) TMB. (C) MSI. *, P<0.05, **, P<0.01. TMB, tumor mutational burden; MSI, microsatellite instability; TCGA, The Cancer Genome Atlas.

positively associated with BRCA, DLBC, GBM, HNSC, KICH, SKCM, STAD, and UCEC in TMB, but negatively associated with LUAD (*Figure 3B*, Table S2). The expression of *HINT1* was negatively correlated with MSI in KIRC, but positively correlated with HNSC, LUSC, PAAD, and STAD (*Figure 3C*, Table S3).

Stromal scores revealed that *HINT1* was positively correlated with GBMLGG and KIPAN, but negatively correlated with LGG, UCEC, LUAD, SARC, COAD, COADREAD, PRAD, HNSC, LUSC, THYM, LIHC, SKCM, BLCA, THCA, MESO, READ, OV, PAAD, LAML, and ACC (*Figure 4*). The immune scores showed that *HINT1* was positively correlated with GBM, GBMLGG, and KIPAN, but negatively correlated with LGG, BRCA, LUAD, ESCA, SARC, COAD, COADREAD, PRAD, LUSC, THYM, SKCM, THCA, READ, PAAD, TGCT, LAML, and ACC (*Figure 5*). The estimate scores confirmed that *HINT1* was positively associated with GBM and GBMLGG, but negatively associated with LGG, UCEC, BRCA, LUAD, SARC, COAD, COADREAD, PRAD, HNSC, LUSC, THYM, SKCM, THCA, READ, OV, PAAD, TGCT, LAML, and ACC (*Figure 6*).

Expression pattern of HINT1 in single-cell and its relationship with tumor functional status

Due to the complexity of tumor cells, single-cell transcriptomic sequencing is essential for the analysis of diverse cancer cells, immune cells, endothelial cells, and stromal cells (20). We used the CancerSEA database to determine whether *HINT1* plays a role in tumorigenesis at single-cell levels across a variety of cancers. As *Figure 7A* and Table S4 show, *HINT1* was significantly positively correlated with the cell cycle, DNA repair, and invasion, and significantly negatively correlated with angiogenesis, apoptosis, differentiation, hypoxia, inflammation, metastasis, and quiescence in AML; *HINT1* expression in GBM was significantly correlated with cell cycle, DNA damage, DNA repair, and invasion, and significantly negatively correlated with angiogenesis, differentiation, hypoxia, inflammation, and quiescence; *HINT1* was significantly positively correlated with cell cycle, DNA damage and DNA repair, and significantly negatively correlated with angiogenesis, differentiation, inflammation, metastasis, quiescence, and stemness in LUAD; *HINT1* expression was significantly positively correlated with angiogenesis, apoptosis, differentiation, EMT, hypoxia, invasion, metastasis, and stemness in renal cell carcinoma (RCC); *HINT1* was significantly positively related with apoptosis, cell cycle, DNA damage, DNA repair and invasion, and significantly negatively correlated with angiogenesis, differentiation, inflammation, and quiescence in BRCA; *HINT1* expression was significantly positively correlated with angiogenesis, cell cycle, differentiation, DNA damage, DNA repair, EMT, hypoxia, invasion, metastasis, and proliferation in HNSC, and significantly negatively associated with quiescence and stemness.

Oncogenic role of HINT1 in BC cells

The above single-cell sequencing results suggested that *HINT1* expression was closely related to tumor apoptosis; however, we focused on the effect of *HINT1* on the apoptosis of the MCF-7 cells. The annexin V/PI analysis showed that in the MCF-7 cells, after the upregulation

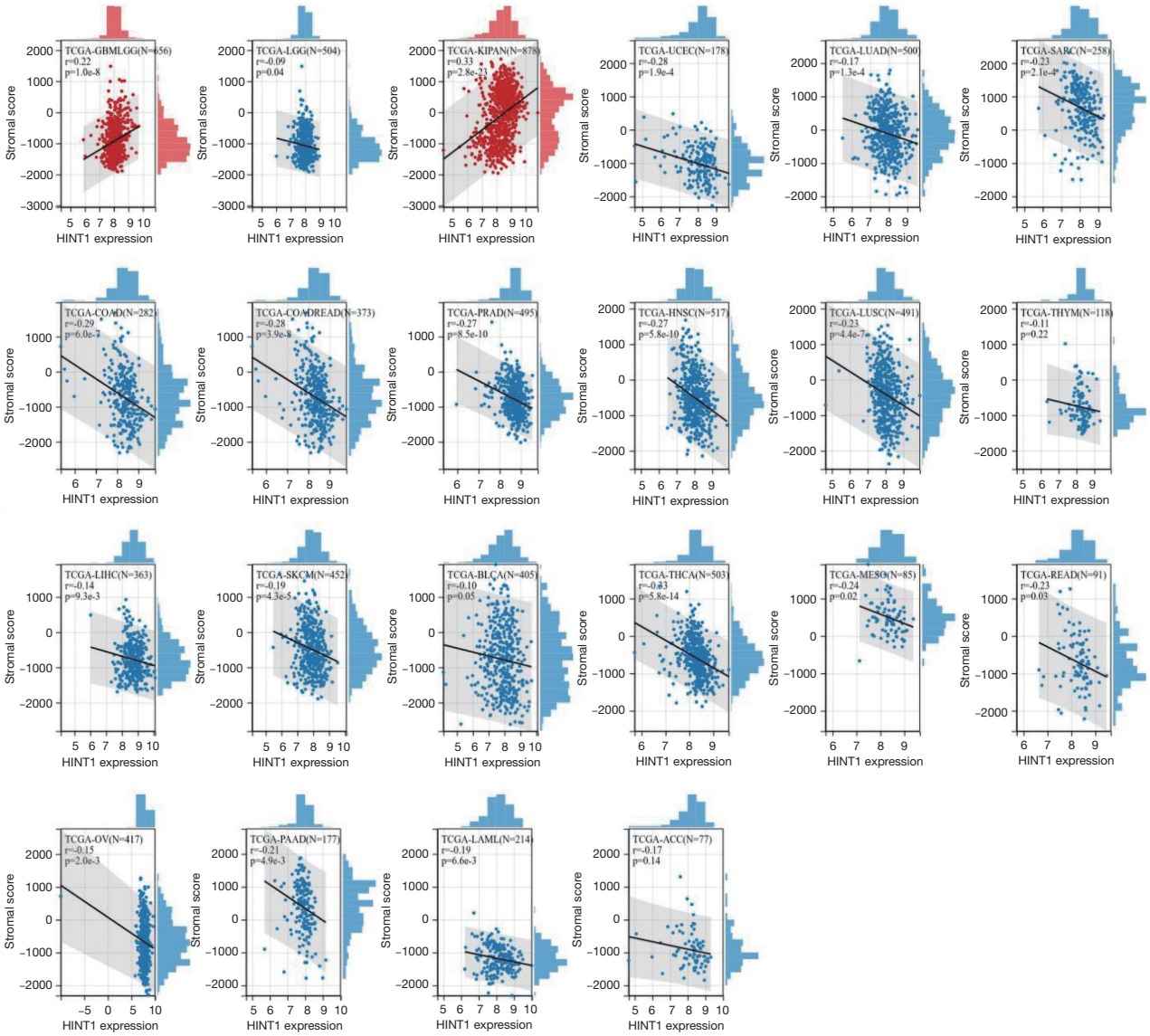

Figure 4 Association between *HINT1* gene expression and stromal scores in 33 different cancer types.

of *HINT1*, the number of apoptotic cells (6.61%) was significantly increased compared to that of the control group (4.22%) (P<0.05) (*Figure* 7*B*,7*C*).

It was previously reported that *HINT1* inhibited *MITF* and β-catenin expression in several cancer types (9). A western blot was conducted using MCF-7 cells expressing *HINT1* overexpression to determine whether *HINT1* could inhibit the expression of *MITF* and β-catenin in BC (*Figure* 7*D*). After the successful transfection of the *HINT1* overexpression plasmids, there was a significant reduction in the expression of *MITF* (P=0.0449, *Figure* 7*E*) and β-catenin (P=0.0008, *Figure* 7*F*) in comparison to the control groups.

To evaluate the effect of *HINT1* on the Akt pathways, western blotting was performed on the *HINT1*-overexpressed MCF-7 cells (*Figure* 7*G*). As a result of the overexpression of *HINT1*, the phosphorylation of Akt (p-Akt) was significantly decreased, while the expression of Akt did not show any significant change overall (P=0.6413, *Figure* 7*H*).

Discussion

The cancer suppressor gene *HINT1* is part of the *HIT* gene family, and has been found to be abnormally expressed

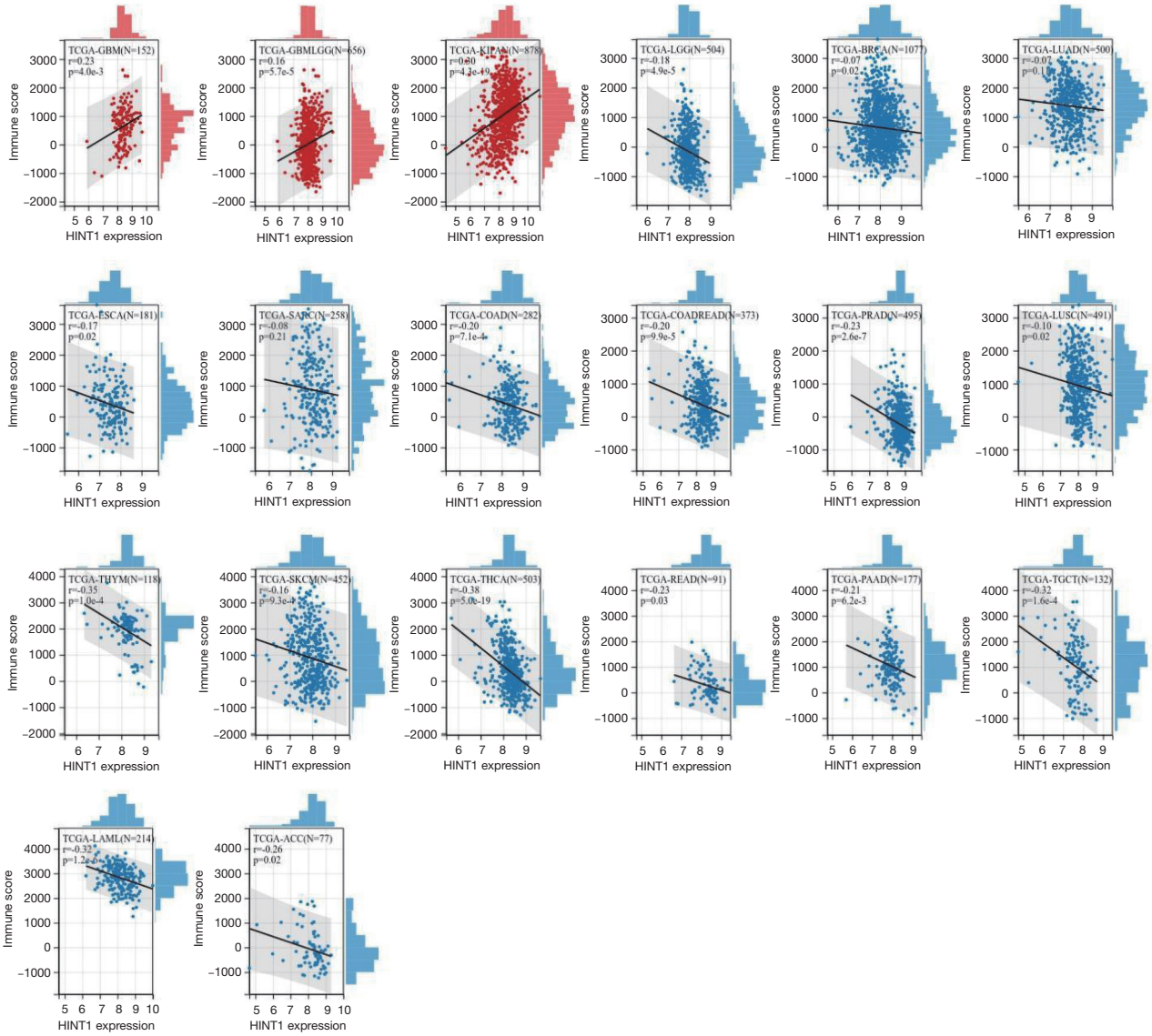

Figure 5 Association between *HINT1* gene expression and immune scores in 33 different cancer types.

in various malignancies, including osteosarcoma, gastric cancer, and prostate cancer (24-26). It was found that *HINT1* overexpression enhanced the cell number in the G0/G1 phases, but reduced the cell number in S and G2/M phases. *HINIT1* also induced the G1 phase arrest to inhibit cell proliferation. In *in-vitro* models, *HINT1* elevation may lead to a reduction in osteosarcoma cell proliferative potential and an increase in apoptosis (26). *HINT1* overexpression increased the number of gastric cancer cells in G0/G1 phase but reduced the number of cells in the S and G2/M phases, thereby inhibiting gastric cancer cell proliferation (27). However, the deletion of *HINT1* expression significantly inhibited gastric cancer cell proliferation and weakened the repair of DNA damage caused by chemotherapy (27). In animal models, both $HINT1^{+/-}$ and $HINT1^{-/-}$ mice displayed a comparable increase in tumor incidence, while the deletion of *HINT1* in mice resulted in greater chances of spontaneous tumor development and greater susceptibility to tumor induction by chemical carcinogens (28,29).

According to TCGA pan-cancer data, the *HINT1* gene differed significantly between BRCA, CHOL, ESCA, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, STAD, COAD, KICH, and READ when compared to the matched

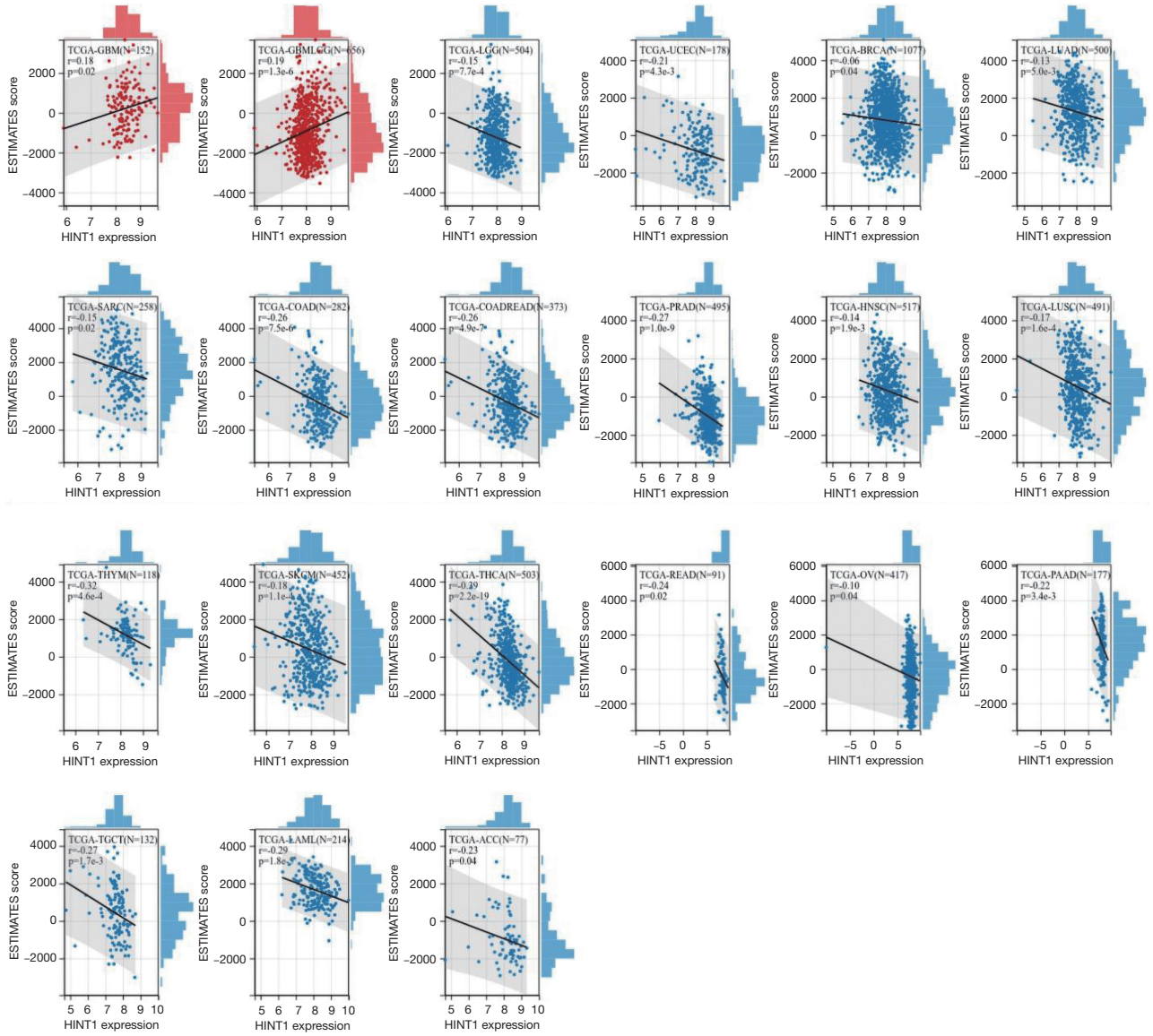

Figure 6 Association between *HINT1* gene expression and estimate scores in 33 different cancer types.

adjacent normal tissues. *HINT1* was associated with risk factors in GBMLGG, LUAD, and KICH, but protective factors in KIRC, MESO, and LAML. Thus, *HINT1* gene expression is an important prognostic biomarker in many cancers, especially LUAD, KICH, and KIRC.

The cancer immunity cycle refers to the body's immune response to cancer (26). It is important to recognize that the activities of the cancer immunity cycle reflect the final effects of complex immunomodulatory interactions in the TME. Immune checkpoint inhibitors have become a great innovation in cancer treatment over the past decade, but not all patients will benefit from immunotherapy (30). According to our results, there were significant correlations between *HINT1* and TMB and MSI in different types of tumors, which suggests that *HINT1* may be used as a screening tool to determine a dominant population in immunotherapy.

In the tumor types of BRCA, DLBC, GBM, HNSC, KICH, SKCM, STAD, and UCEC, high levels of *HINT1* were associated with higher TMB, predicting a good response to immunotherapy. We also observed positive correlations between *HINT1* and MSI in HNSC, LUSC, PAAD, and STAD, and negative correlations in KIRC.

The TME, including immune cells and stromal cells, has

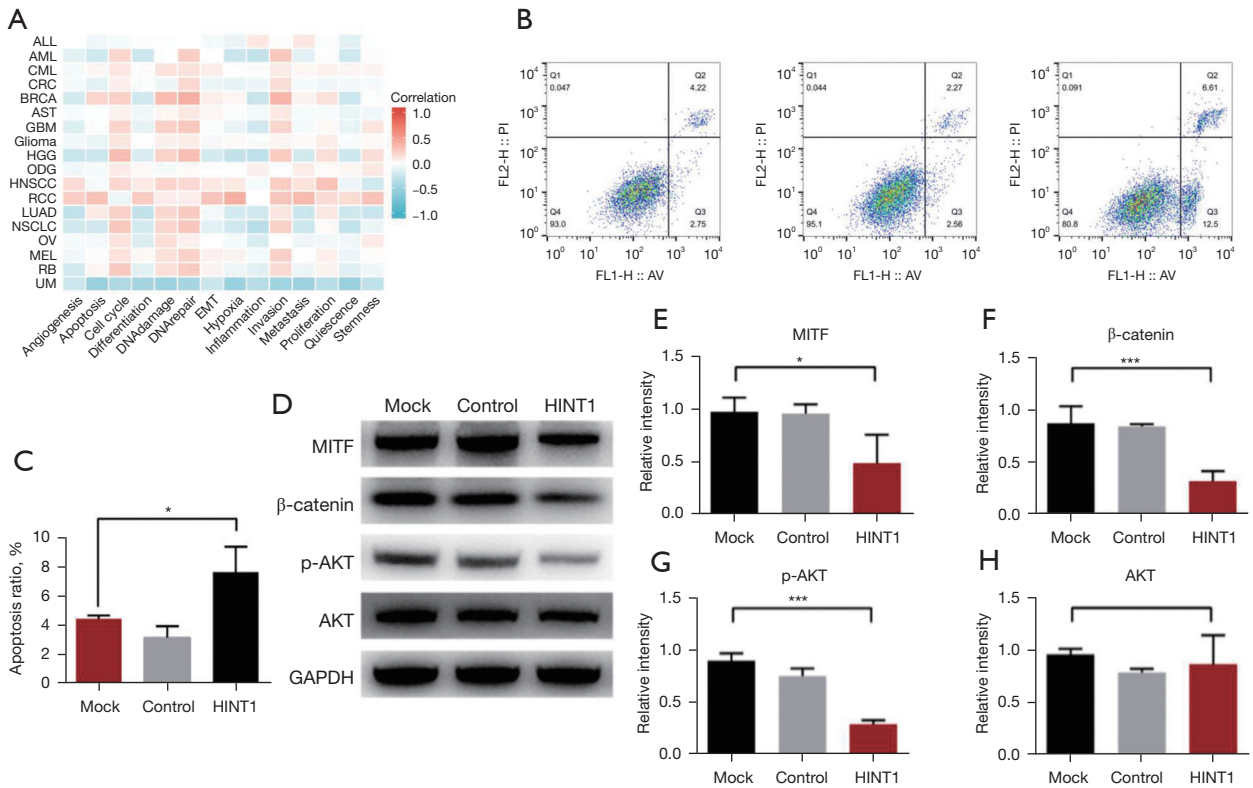

Figure 7 Oncogenic role of *HINT1* in breast cancer cells. (A) Expression pattern of *HINT1* in single-cell and its relationship with tumor functional status. (B,C) The effect of *HINT1* on apoptosis in MCF-7 cells. (D-H) The protein expression levels of *MITF*, β-catenin, p-Akt, and Akt were measured by western blotting. *, P<0.05, ***, P<0.001. GAPDH was used as the loading control. MCF-7, Michigan Cancer Foundation-7; *MITF*, microphthalmia transcription factor; p-Akt, phosphorylation of protein kinase B; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

been shown to be closely related to immunotherapy (31,32). The present study found negative correlations between the *HINT1* gene expression and immune, stromal and estimate scores in the majority of the 33 TCGA tumors, which suggests that *HINT1* may be involved in tumor progression by modulating the immune microenvironment. In the course of cancer growth, metastasis and drug resistance are involved in the development of stromal and immune cells. Our findings suggested that *HINT1* plays an important role in regulating tumor behavior by interacting with TME. Boieri *et al.* demonstrated that TSLP-stimulated CD4$^+$ T cell immunity can block breast cancer growth by inducing a cellular senescent phenotype in advanced breast tumors. The tumor-suppressive phenotype is mediated by cellular senescence, in which effector CD4$^+$ T cells can directly block advanced breast tumor development (33). In the present study, the *HINT1* gene played an important role in tumor immunity escape, as shown by the close correlation between *HINT1* and CD4$^+$ T cell infiltration. Thus, the relationship between *HINT1* and other immune cells needs to be further investigated, and *HINT1* may become a new biomarker for the screening of the dominant population of immunotherapy.

According to this study, *HINT1* played an important role in the suppression of BC cells. The MCF-7 BC cell line showed increased apoptosis when *HINT1* was overexpressed. Research has shown that tumor progression can be stopped by the process of apoptosis, a programmed cell death mechanism that eliminates damaged and malignant cells (34). A study also showed that *HINT1* promotes the apoptosis of damaged cells, thereby inhibiting gastric cancer progression (27). In a human colon cancer SW480 cell line, *HINT1* was shown to promote cell apoptosis by upregulating protein 53 and inhibiting activator protein 1 transcription factors, which in turn inhibits the proliferation and angiogenesis of rectal cancer cells (2). These results

indicated that inducing cell apoptosis by *HINT1* represents a promising approach for BC treatment.

MITF is a master regulator of the expression of various genes related to survival, metastasis, cell cycle arrest and differentiation. *MITF* is also expressed in other cell types, including osteoclasts, mast cells, B cells, and natural killer (NK) cells (35). To date, *HINT1* has been shown to be immunologically related to several other immunosuppressive pathways, including the catenin, *MITF* and p-Akt pathways (6,7). It has been reported that these oncogenic pathways impair the infiltration of tumor-infiltrating immune cells by reducing their expression of immunomodulators (36,37).

Conclusions

In conclusion, the present study showed that *HINT1* plays an oncogenic role in various cancers, and it could also be used as a biomarker for BC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6637/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6637/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 The correlation between the HINT1 mRNA expression and stemness in the TCGA pan-cancer database

Tumor type	Cor	P-value	
ACC	0.263	0.022	*
BLCA	0.199	0	***
BRCA	0.065	0.026	*
CESC	0.127	0.026	*
CHOL	0.051	0.738	
COAD	0.153	0.006	**
DLBC	0.303	0.038	*
ESCA	0.312	0	***
GBM	0.171	0.026	*
HNSC	0.331	0	***
KICH	-0.49	0	***
KIRC	0.081	0.049	*
KIRP	0.093	0.097	
LAML	0.101	0.193	
LGG	0.238	0	***
LIHC	0.302	0	***
LUAD	0.222	0	***
LUSC	0.38	0	***
MESO	0.304	0.004	**
OV	0.156	0.007	**
PAAD	0.119	0.131	
PCPG	0.176	0.017	*
PRAD	0.29	0	***
READ	0.181	0.075	
SARC	0.351	0	***
SKCM	0.15	0.001	**
STAD	0.201	0	***
TGCT	–0.009	0.913	
THCA	0.112	0.008	**
THYM	–0.071	0.439	
UCEC	0.096	0.176	
UCS	0.195	0.145	
UVM	0.216	0.056	

*P<0.05, **P<0.01, ***P<0.001.

Table S2 The correlation between the HINT1 mRNA expression and TMB in the TCGA pan-cancer database

Tumor type	Cor	P-value	
ACC	0.063	0.587	
BLCA	0.021	0.669	
BRCA	0.064	0.038	*
CESC	0.105	0.183	
CHOL	0.141	0.411	
COAD	0.045	0.462	
DLBC	0.412	0.004	**
ESCA	0.036	0.641	
GBM	0.179	0.023	*
HNSC	0.1	0.026	*
KICH	0.249	0.046	*
KIRC	0.071	0.173	
KIRP	0.046	0.442	
LAML	-0.14	0.108	
LGG	0	0.992	
LIHC	0.077	0.165	
LUAD	-0.226	0	***
LUSC	0.084	0.07	
MESO	0.056	0.615	
OV	-0.052	0.382	
PAAD	-0.044	0.562	
PCPG	0.021	0.782	
PRAD	0.027	0.549	
READ	0.029	0.797	
SARC	0.013	0.836	
SKCM	0.107	0.021	*
STAD	0.298	0	***
TGCT	0.05	0.538	
THCA	-0.066	0.142	
THYM	0.129	0.163	
UCEC	0.258	0.001	**
UCS	0.142	0.293	
UVM	-0.046	0.69	

*P<0.05, **P<0.01, ***P<0.001.

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Table S3 The correlation between the HINT1 mRNA expression and MSI in the TCGA pan-cancer database

Tumor type	Cor	P-value	
ACC	0.142	0.217	
BLCA	-0.002	0.963	
BRCA	-0.017	0.584	
CESC	0.087	0.13	
COAD	0.011	0.865	
ESCA	0.066	0.363	
GBM	–0.104	0.189	
HNSC	0.151	0	***
KIRC	–0.098	0.038	*
KIRP	0.103	0.069	
LGG	0.068	0.123	
LIHC	–0.016	0.75	
LUAD	0.008	0.847	
LUSC	0.106	0.027	*
OV	0.058	0.306	
PAAD	0.178	0.02	*
PCPG	0.053	0.485	
PRAD	0.045	0.297	
READ	-0.054	0.629	
SKCM	0.097	0.316	
STAD	0.293	0	***
THCA	0.042	0.322	
UCEC	0.395	0.145	

*P<0.05, ***P<0.001.

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Table S4 Expression pattern of HINT1 in single cell and its relationship with 14 tumor functional status

	Angiogenesis	Apoptosis	Cell Cycle	Differentiation	DNAdamage	DNArepair	EMT	Hypoxia	Inflammation	Invasion	Metastasis	Proliferation	Quiescence	Stemness
ALL	-0.009	-0.062	-0.044	0.01	-0.012	0.002	-0.051	-0.099	0.088	0.014	0.099	-0.027	-0.137	-0.01
AML	-0.139	-0.219	0.176	-0.242	-0.005	0.222	-0.006	-0.256	-0.297	0.257	-0.138	-0.014	-0.223	0.01
CML	-0.042	0.027	0.097	0.01	0.118	0.117	0.068	-0.003	-0.015	0.084	0.033	0.049	0.028	0.042
CRC	-0.044	-0.124	0.064	-0.028	-0.071	0.165	-0.085	-0.113	-0.072	0.14	-0.041	-0.064	-0.055	-0.042
BRCA	-0.194	0.178	0.212	-0.226	0.317	0.388	0.064	0.037	-0.257	0.354	0.047	0.121	-0.236	-0.021
AST	-0.034	-0.01	0.048	-0.067	0.037	0.091	0.036	-0.02	-0.061	0.092	-0.044	-0.036	-0.093	-0.068
GBM	-0.15	-0.016	0.186	-0.11	0.211	0.256	-0.026	-0.097	-0.213	0.178	-0.041	0.021	-0.104	0.105
Glioma	-0.057	-0.088	0.106	-0.002	0.05	0.074	-0.04	0.017	-0.024	0.035	0.016	0.069	-0.023	0.026
HGG	-0.268	-0.242	0.307	-0.17	0.198	0.3	-0.195	-0.292	-0.314	0.234	-0.203	0.134	-0.232	0.133
ODG	-0.058	-0.039	0.083	0.024	-0.03	0.018	-0.012	0.002	0.053	-0.002	0.071	0.016	0.037	0.088
HNSCC	0.149	-0.033	0.167	0.078	0.187	0.163	0.092	0.089	-0.038	0.225	0.115	0.284	-0.081	-0.219
RCC	0.222	0.286	-0.066	0.234	0.073	0.066	0.266	0.387	-0.003	0.265	0.34	0.149	0.148	0.283
LUAD	-0.294	0.078	0.2	-0.288	0.166	0.236	-0.048	-0.197	-0.216	0.104	-0.256	0.094	-0.231	-0.254
NSCLC	-0.225	-0.216	0.255	-0.192	0.18	0.26	-0.13	-0.223	-0.342	0.206	-0.243	0.014	-0.294	-0.077
OV	-0.042	-0.052	0.108	-0.159	0.136	0.052	0.003	-0.051	-0.183	-0.009	-0.081	-0.066	-0.067	0.083
MEL	-0.055	0.01	0.114	0.026	0.128	0.199	0.05	0.048	-0.044	0.202	0.002	0.06	-0.081	0.007
RB	-0.201	0.027	0.246	-0.122	0.13	0.234	0.048	-0.07	-0.131	0.152	-0.073	0.049	-0.142	-0.062
UM	-0.243	-0.53	-0.4	-0.466	-0.508	-0.456	-0.254	-0.523	-0.365	-0.479	-0.513	-0.347	-0.527	-0.306

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